Short Communication

Compound heterozygosity for *TNXB* genetic variants in a mixed-breed dog with Ehlers-Danlos syndrome

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Running title: *TNXB* variants in canine Ehlers-Danlos syndrome

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Summary

The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of inherited connective tissue disorders characterized by skin hyperextensibility, joint hypermobility and tissue fragility. Inherited disorders similar to human EDS have been reported in different mammalian species. In the present study, we investigated a female mixed-breed dog with clinical signs of EDS. Whole genome sequencing of the affected dog revealed two missense variants in the *TNXB* gene, encoding the extracellular matrix protein tenascin XB. In humans, *TNXB* genetic variants cause classical-like EDS or the milder hypermobile EDS. The affected dog was heterozygous at both identified variants. Each variant allele was transmitted from one of the case's parents, consistent with compound heterozygosity. While one of the variant alleles, XM_003431680.3:c.2012G>A, p.(Ser671Asn), was private to the family of the affected dog and absent from whole genome sequencing data of 599 control dogs, the second variant allele, XM_003431680.3:c.2900G>A, p.(Gly967Asp), was present at a low frequency in the Chihuahua and Poodle population. Given that *TNXB* is a functional candidate gene for EDS, we suggest that compound heterozygosity for the identified *TNXB* variants may have caused the EDS-like phenotype in the affected dog. Chihuahuas and Poodles should be monitored for EDS cases, which might confirm the hypothesized pathogenic effect of the segregating *TNXB* variant.

Keywords: skin, genodermatosis, whole genome sequencing, precision medicine, Canis lupus familiaris, connective tissue, animal model

The Ehlers-Danlos syndromes (EDS) are a heterogeneous group of inherited connective tissue disorders (Beighton et al. 1998). The main characteristics of these disorders are skin hyperextensibility, joint hypermobility and tissue fragility. In humans, there are 13 EDS subtypes (Malfait et al. 2017). Connective tissue disorders resembling human EDS were further reported in mammals such as cats, cattle, dogs, horses, minks, rabbits and sheep (Colige et al. 1999; Harvey et al. 1990; Hegreberg et al. 1969; Monthoux et al. 2015; Paciello et al. 2003; Sequeira et al. 1999; Spycher et al. 2018; Tryon et al. 2007; Zhou et al. 2012).

Historically, it was assumed that EDS is caused by genetic variants in genes encoding fibrillary collagen or collagen modifying proteins. However, more recently, variants in additional genes were identified that at first sight are not directly involved in collagen structure or synthesis (Malfait et al. 2017). One of these genes is *TNXB*, encoding the large extracellular matrix glycoprotein tenascin XB (Bristow et al. 1993; Burch et al. 1997). Tenascin XB was shown to regulate collagen deposition by dermal fibroblasts (Mao et al. 2002). In humans, variants in *TNXB* cause a rare monogenic autosomal recessive subtype of EDS, the classical-like EDS (OMIM 606408). Characteristic for this type are hyperextensible and fragile skin as well as hypermobile joints, but compared to the classical EDS, affected individuals do not show atrophic scarring (Schalkwijk et al. 2001). Tenascin XB haploinsufficiency in individuals heterozygous for *TNXB* genetic variants may, among other genetic defects, also cause another milder form of Ehlers-Danlos syndrome, the hypermobility type (Zweers et al. 2003). Furthermore, *TNXB* genetic variants were reported to cause vesicoureteral reflux 8, in an autosomal dominant manner (OMIM 615963; Gbadegesin et al. 2013; Elahi et al. 2016).

In the present study, we investigated a female dog with clinical signs of EDS. The dog was referred because of fragile skin that teared or bruised easily even from minor injuries, leading to severe wounds that healed poorly. According to the owner, the skin fragility had been present since the dog was a puppy. At the time of examination, the dog was 21 months old and appeared in a good general health condition. Neither wounds nor scars were present but the skin was hyperextensible (Figure 1). The affected dog was of mixed breed and its parents as well as the only known littermate were clinically normal.

We prepared a PCR-free DNA library of the affected dog and collected 175,177,490 read pairs (2 x 150 bp) or roughly 20x coverage on an Illumina HiSeq 3000 instrument (ENA project accession PRJEB16012, sample accession SAMEA4867919). Variants were called with respect to the reference genome assembly CanFam 3.1 and compared to 8 wolf and 591 dog genomes of genetically diverse breeds as described previously (Table S1; Bauer et al. 2018) Given that the mode of inheritance was unknown, we filtered for variants that were present in either a heterozygous or homozygous state in the affected dog and absent in the control genomes, assuming that the causative variant is rare and, in our study sample, only present in the affected dog. Applying a hard filtering approach, we detected 48 variants predicted to be protein-changing (Table S2). Three of them were present in a homozygous state in the affected dog, but none of them was located in a functional candidate gene for EDS. Of the 45 private heterozygous protein changing variants, one was a missense variant in the *TNXB* gene (XM_003431680.3:c.2012G>A, p.(Ser671Asn)). Given that the human classical-like type of EDS caused by *TNXB* variants is inherited in a monogenic autosomal recessive mode, we hypothesized that the identified variant could be causative for the EDS phenotype in compound heterozygosity together with another variant in *TNXB*. We therefore extracted all variants in the genomic region of *TNXB* ± 5 kb from the vcf-file including variants in the affected dog and all 599 control genomes (Table S3). These variants were then filtered for protein-changing variants present with a heterozygous genotype in the affected dog that were not present in a homozygous state in any of the control dogs. Within the analysed ~67 kb interval, there were only two such variants, one of them being the previously identified missense variant private to the affected dog. The second variant was a missense variant, XM_003431680.3:c.2900G>A, p.(Gly967Asp), which was also present in a Chihuahua. To investigate how the two variants segregated in the family of the affected mixed breed dog, we extracted genomic DNA from EDTA blood of the parents and the littermate and genotyped the dogs with the Sanger method. Both parents were heterozygous for one of the variants, and only the affected dog carried both variants in a heterozygous state consistent with compound heterozygosity. The non-affected littermate was homozygous wildtype at c.2012G>A and heterozygous at c.2900G>A

(Figure 2). We additionally genotyped 603 dogs from 69 different breeds for both variants using the Sanger method. All tested dogs were homozygous for the wildtype allele at c.2012G>A. At c.2900G>A, 601 dogs were homozygous for the wildtype allele. One Chihuahua and one Poodle were heterozygous for this variant (Table S3).

To the best of our knowledge, EDS due to *TNXB* genetic variants has only been reported in humans and mice, but no domestic animal species. In humans, most known cases were caused by complete absence of tenascin XB due to degradation of the mutant transcript resulting from frameshift, nonsense or large deletion variants (Schalkwijk et al. 2001). However, in some patients, missense variants were also reported to cause the classical-like EDS phenotype (Pénisson-Besnier et al. 2013; Kaufman and Butler, 2016). The variants identified in the affected dog both lead to amino acid substitutions: p.(Ser671Asn) is located in one of multiple EGF-like domains, p.(Gly967Asp) in one of multiple fibronectin type 3 modules. The p.(Ser671Asn) variant affects a serine residue in the canine TNXB protein which is not conserved across species. The corresponding amino acid in human, rhesus macaque and chimpanzee at the homologous position is a glycine. Given the different chemical properties and the larger size of asparagine, it seems conceivable that a substitution from glycine to serine but not to asparagine might be tolerated. The second variant, p.(Gly967Asp), affects a glycine residue which is conserved across mammals. It is unclear, whether these amino acid substitutions affect protein folding or function.

Based on the known function of *TNXB* and its role in human EDS, the rarity of the two identified missense variants in dogs, and the co-segregation of the mutant alleles with the disease phenotype in the family of the affected dog, we suggest that EDS in this dog may have been caused by compound heterozygosity for the c.2900G>A and c.2012G>A variants. Given that this is a single case investigation and that we have no functional confirmation of a tenascin XB deficiency, this result must be considered preliminary and should be interpreted with caution. We also have to stress that our analysis was not suitable to detect large structural variants. While one of the variants was only identified in the family of the affected crossbred dog, the c.2900G>A variant segregated at low frequency in Chihuahuas and Poodles. Therefore, the

Chihuahua and Poodle breeds should be carefully monitored for cases of EDS. If dogs with recessively inherited EDS occur and turn out to be homozygous for the ^{967}Asp allele, then the pathogenicity of the variant would be confirmed and implementation of genetic testing to avoid future carrier x carrier matings is indicated.

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Figure 1. Mixed breed female dog with clinical signs of Ehlers-Danlos syndrome. Skin hyperextensibility is shown at different anatomical locations.

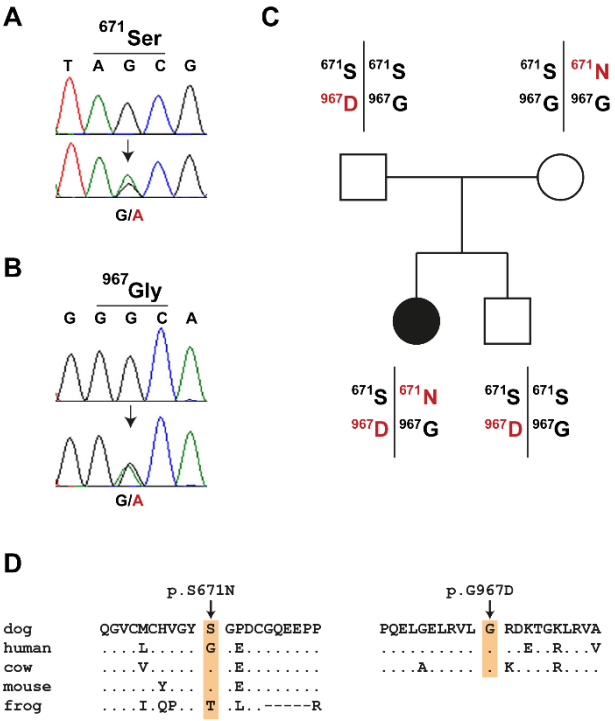

Figure 2. Details of the detected *TNXB* variants. (A, B) The identified c.2012G>A and c.2900G>A variants were confirmed by Sanger sequencing. (C) Pedigree of the investigated family of mixed breed dogs. Phased haplotypes with the alleles at the two variants are given for each animal. Only the affected dog is compound heterozygous and carries mutant alleles on both copies of the *TNXB* gene. (D) Multiple species amino acid alignments in the region of the variants. The following protein accessions were used: XP_003431728.2 (dog), NP_061978.6 (human), NP_777128.1 (cow), NP_112453.2 (mouse), XP_002941313.2 (*X. tropicalis*). The TNXB protein has a highly repetitive domain structure with 19 EGF-like domains and 30 fibronectin type III repeats in humans. The exact number of these repeats is somewhat variable between species. The p.(Gly967Asp) variant affects the first fibronectin type III repeat, which is missing from the mouse and frog orthologs.

Supplementary Material

Table S1. Information on 592 dog and 8 wolf genome sequences.

Table S2. Private protein-changing variants detected by whole genome sequencing.

Table S3. Sequence variants and genotypes in the region of the *TNXB* gene.

Table S4. Genotypes of 603 control dogs from 69 various other breeds.